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DECIPHERING THE ROLE OF GENE EDITING: RETORT FOR PERPLEXED UNHEALTHY AND DISEASED CONDITIONS

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Abstract

A breakthrough approach that unleashed the function of bacterial nucleases and the engineered ones has prompted the Creation of innovative gene editing technologies and procedures. In practically all eukaryotic cells, puzzled unclear Situations can be resolved by directly addressing certain genomic sequences and modifying them for use in various tactics. This expanding field of research has made this possibility possible. The ability to detect unique phenomena linked to the Genetic and epigenetic variables driving the development of disease have been enhanced through genome editing. It has majorly promoted the way for creating more precise cellular and animal models for elucidating pathological pathways and has shown remarkable potential in varying fields such as biotechnology, crop engineering, and biomedical science research Is under way. The creation of sophisticated methods based on nucleases, such as those connected to the CRISPR system, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), and CRISPR-Cas-Associated nucleases, has greatly enhanced their practical utility in designing most promising biotechniques. Surprisingly, The applications of genome editing are leading to a variety of therapeutic and therapy options. This review will focus on Some applications and research that have revealed their implementation and strategies.

Keywords: Epigenetic, Biotechnology, CRISPR, ZFN, TALENs, Nucleases.

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Introduction

The development of novel strategies and measures has always been in substantial demand to understand vital kinks in human, animal, and environmental health. The gross changes in the genome of different microorganisms have raised significant concerns about the advent of critically harmful pathogens that are terminating in the birth of various diseases. The stories of great discoveries and inventions frequently depict a string of fortunate coincidences. Scientific advancements in different aspects of biotechnological tools, techniques, and processes are always in the spotlight for the evolution of several resolutions. Gene editing has served as a boon for culminating various pathogenic effects. In this era of research, scientists are converging different and vast practices to make a remarkable impact on scientific approaches. Gene editing is one of the spectacular approaches that has astonished biotechnological methods with startling results in various domains such as food, agricultural, medical, plant, and human health. This article will give a general overview of the field of genome editing and the tools that are used to modify genome sequences, starting with a brief history of the field and concentrating on key developments along the way.

Journey of genome-editing-

The genome of the eukaryotic system is complex and intricate. The urge to understand the basic phenomenon and metabolic activities of a cell have forced researchers to fabricate different methods for treating diseased conditions. This intervention provoked a step towards target-specific gene manipulations, and thus the technique of genome editing came under effect. The discovery of restriction enzymes (Danna *et al.*, 1971; Kelly *et.al.*, 1971; Smith *et.al.*, 1971) turned the enigma surrounding bacteria's defense mechanism against bacteriophages. It was the first significant development in genetic engineering that made it possible for experts to accurately change DNA sequences using molecular scissors. It further emphasized the role of gene manipulation studies in mammalian cells. The remarking contribution was the studies conducted by Smithies, and coworkers which proved that the exogenous DNA copies get incorporated into the host genome via a process of homologous recombination (Capecchi *et.al.*, 1989 Thomas *et.al.*, 1986 Smithies *et al.*, 1985). However, the extent of incorporation may vary accordingly as the cell can present itself in different stages.

Nucleases as the executor of genome editing

One of the earliest discoveries was the finding that when a double-strand break (DSB) is introduced at a target, the frequency of targeted gene integration increases location can be measured in several orders of magnitude (Rouet *et.al.*, 1994; Rudin *et.al.*, 1998). As a result, numerous research teams concentrated on creating various methods for

achieving specific DSBs. Early studies involved inserting specific DSBs into the mouse genome with the help of unusual cutting endonuclease enzymes, including the 18-bp cutter I-SceI (Rouet *et al.,* 1994). The process includes the usage of meganucleases (long segments of 14–40 bp DNA are recognized by endonucleases.), which in turn enhanced genome-editing efficiency. Despite this efficacy, Due to certain restrictions, this technique was limitedThe likelihood of discovering a meganuclease that specifically targets a desired site remained low since double-strand breaks (DSBs) are repaired by the fragile non-homologous end joining (NHEJ) DNA repair pathway Which may arbitrarily add or remove DNA fragments at the break sites, preventing exogenous DNA template from incorporating into the DSBs (Jeggo, P. A. 1998). Scientists started manipulating naturally occurring meganucleases to overcome these challenges (Rosen *et al.* 2006, Sussman *et al.*, and 2004 Seligman et al. 2002). It was probably impossible to find a meganuclease that can target genes at the genomic level despite all of this effort.

The identification and use of eukaryotic zinc finger proteins have significantly advanced this goal. Zinc ions are used to selectively link small protein motifs known as zinc fingers to DNA sequences. Each zinc finger pattern may identify a DNA sequence as short as 3 base pairs (Klug, A., and Rhodes, D. 1987). Press of Cold Spring Harbor Thus, a multi-zinc finger motif complex, in contrast to meganucleases, could achieve more DNA binding selectivity. The discovery of the Fok I endonuclease led to the synthesis of programmable nuclease proteins by combining zinc finger proteins with the DNA cleavage domain of this enzyme (Kim *et.al.,* 1996).

Numerous studies (Bibikova *et al.,* 2001; Kim *et al.,* 1996) revealed that zinc finger nucleases (ZFNs) greatly speed up targeted homologous recombination in both model species and human cells. With the development of ZFNs, it became much easier to accurately alter the genomes of living cells at site-directed locations, which also made it possible to apply these technologies to treat disease (Urnov et al. 2005; Porteus *et al*., 2003). Each zinc finger recognised a 3-bp DNA code, allowing a combinatorial assembly of 6-7 zinc fingers to selectively target any 18–21 bp genomic sequences (Miller et al., 2007). There are 64 different zinc fingers in the pool, making up 43 combinations. As proteins from Xanthomonas bacteria can preferentially detect one single base instead of three bases as with ZFNs, another group of nucleases known as transcription activator-like effector (TALE) attracted increased interest. Scientists were drawn to this newly discovered class of proteins in order to do more noteworthy research on them (Urnov *et al*., 2010, Boch *et al.,* 2009).

A more potent class of proteins known as TALEN was discovered by fusing the Fok I DNA cleavage domain with TALE nucleases (Li *et. al.* 2011, Miller *et. al.,* 2011, Zhang *et. al.,* 2011, Moscou *et. al*., 2009).

The miraculous onset of genomic manipulation technique: CRISPR

In addition to the previously stated nucleases, new proteins must be designed or engineered in order to target diverse regions of the genome. Sequentially increasing the effectiveness of genome editing was the discovery of synthetic meganucleases, ZFNs and TALENs. However, ZFNs and TALENs were not widely employed by the scientific community due to the difficulties involved in their cloning and protein engineering. In this aspect, CRISPR has fundamentally altered the field. More importantly, using it is much simpler and more flexible. The CRISPR gene-editing mechanism is more particular and exact in terms of its DNA-targeting specificity thanks to the meticulous endonuclease activity associated with the CRISPR protein. Additionally, a short guide RNA can regulate the endonuclease activity, increasing its selectivity.

CRISPR stands for clustered regularly interspaced short palindromic repeat DNA sequences. The repeating sequences in Escherichia coli were initially found by Dr. Nakata's team, even though the word CRISPR was not coined until much later (Jansen *et al*., 2002). The CRISPR repeat clusters were interestingly separated by spacers, which are non-repeating DNA sequences, in contrast to the genome's traditional tandem repeats. Researchers spent more than ten years determining the nature and origin of these spacer sequences. As a part of the human genome project (HGP), the genomes of several more animals, including numerous different phages, were also sequenced. Through computational analysis of these genomic sequences, researchers identified key properties of CRISPR repeat and spacer elements. To start, 90% of archaea and more than 40% of sequenced bacteria, respectively, contain CRISPR sequences (Mojica *et al.*, 2000).

The CRISPR-associated (CAS) genes28 are a collection of well-conserved genes that are situated near to the CRISPR elements. Finally and most intriguingly, the non-repeating spacer DNA sequences were determined to be components of viruses and other mobile genetic elements (Pourcel *et al*. 2005; Bolotin *et al*. 2005; Mojica *et al.* 2005). These findings sparked a great deal of scientific curiosity about the mechanical and functional significance of these CRISPR sequences. Though the particular mode of action was unknown, researchers started to hypothesise that it might serve as a bacterial immune system. The most significant experimental results regarding the possible functionality of CRISPR systems were supplied by the study conducted by Horvath and colleagues.

Significantly, the targeting specificity of the CAS enzymes that provide defence against the phage is determined by the CRISPR spacer sequences (Barrangou *et al*., 2007). These findings further streamlined the CRISPR technique by employing just one short RNA. The endogenous CRISPR system needs the mature crRNA and the trans-activating crRNA (tracrRNA), two short RNAs. Together with the tracrRNA, the crRNA forms a base pair and acts as a guide sequence. For the Cas9 protein-RNA complex to be assembled, which causes double-strand breaks (DSBs) in DNA at specific locations, both crRNA and tracrRNA are required. In example, Jinek et al. demonstrated that a chimeric RNA known as a single guide RNA (sgRNA) was created by merging tracrRNA with crRNA May also drive CRISPR-Cas9 (Jinek *et. al.*, 2012).

In the wake of these discoveries, groundbreaking studies showing how CRISPR may be utilised for in vivo genome editing in eukaryotic cells were published (Mali *et.al*., 2013, Cong *et. al.*, 2013, Jinek *et.al*., 2013). For the first time ever, researchers have a highly flexible tool that could be easily directed to target virtually any region of the genome by just making a short sgRNA. Due to its high editing effectiveness

and simplicity, CRISPR technology has quickly gained the support of researchers from a wide range of fields and become the go-to technique for many genome-targeting applications. Notably, it has been the subject of more than 9000 research articles since it was first used as a genome-editing approach in late 2012, and the number of publications seems to be increasing every year.

Exploration of various CRISPR systems requires a thorough understanding of new CAS proteins and their characterisation. The number of studies attempting to re-engineer the already-studied Cas9 proteins has increased as a result of this. The three main objectives of this field of research are to (i) decrease the size of Cas9 nucleases, (ii) increase their fidelity, and (iii) increase the targeting range of Cas9 variants. At numerous universities, there have been major modifications to the standards for targeting specificity and Cas9 PAM Despite the fact that Cas9 protein size reduction has only slightly improved.

Applications of Gene editing in different fields-

1. Manipulation of gene expression-

The nickase Cas9 platform has been used by Nishida *et al.*, 2016, Komor *et al.*, 2016, and Gaudelli *et al.*, 2017) to create editing tools that enable direct C to T or A to G conversion at the target site without DSBs. Recently, Komor *et al.*, 2016 showed that Cas9 nickase coupled to APOBEC1 deaminase enzyme and Uracyl Glycosylase Inhibitor (UGI) protein efficiently changes cytosine to thymine in the target location without producing double-strand DNA breaks. Notably, a transfer RNA adenosine deaminase and nickase Cas9 were combined to create a new base editor (Gaudelli *et al.*, 2017). Direct A-G conversion is achieved at the target sites using this base editor.

The genomic regions that can be targeted have been greatly expanded by these cutting-edge base-editing techniques. To make these systems more adaptable, researchers are modifying them. This CRISPR base editor has been used to change the genetic code and introduce early STOP codons into genes in recent studies Billon *et al.*, 2017, Kuscu *et al.*, 2017, Adli, M. *et al.*, 2018 showed that the STOP codons TGA (opal), TAG (amber), or TAA (ochre) might emerge from changing C to T at the codons CGA (Arg), CAG (Gln), and CAA (Gln). Research using wild-type (WT) Cas9-mediated gene knockout (KO) is a risky and ineffective alternative to the CRISPR-STOP strategy (Kuscu *et al.*, 2017). The activation-induced adenosine deaminase (AID) enzyme has also been fused to the dCas9 enzyme in addition to APOBEC adenosine deaminase (Ma *et al.* 2016, Hess *et al.*, 2016).

The dCas9-AID complex, in particular, becomes a potent local mutagenesis agent and may be employed as a gain of function screening tool when UGI is absent (Gilbert *et al.*, 2013, Ma *et al.*, 2016, Hess *et al.*, 2016).

2. Clinical, diagnostic and therapeutic –

The genome-engineering technology CRISPR/Cas 9 has produced a number of genome and epigenome altering targets concurrently. CRISPR screenings, when combined with a pluripotent stem cell strategy, are especially successful at obtaining differentiated cells from sick people, such as neurons, glial cells, and brain organoids (Kampmann, M. 2020)). The CRISPR tool and its accompanying protein (Cas) system are composed of the CRISPR locus, which has 2-375 repeat sequences and 21–48 bp, 1-374 interspersed spacer sequences (26-72 bp), and Cas genes. This entire system is applied in several cell line models (human isolated pluripotent stem cells (iPSCs)) and animal models to sequence the therapeutic method in various NDDs by killing off and fixing the damaged genes (Singh *et al.*, 2023).

According to reports, CRISPR offers a focused therapy method for conditions including seizures, autism, and cognitive loss that are related to the neuropsychiatric system.The most prevalent hereditary disorders include schizophrenia (SP), anxiety disorders (AD), bipolar disorders (BP), major depressive disorders (MDD), autism spectrum disorders (ASD), and attention deficit hyperactivity disorders (ADHD). The aforementioned illnesses have been associated with several reported defective genes, such as AS3MT, ANK3, and many other genes. These genes are mostly expressed in cellular processes, immunological control, synaptic transmission, and neural activity. The affected individuals can be exposed to CRISPR to fix the genetic alterations. In order to replace the damaged genes with the normally expressed genes, we can use a gene editing tool (Singh *et al.*, 2023).

Recombinant adeno-associated virus (rAAV) vectors are employed for gene augmentation and gene editing for the treatment of inherited liver diseases (Zabaleta *et al*., 2023). This clarifies the function of gene editing techniques in managing the symptoms of numerous disorders.

The CRISPR/Cas9 system has shown promise in the treatment of cancer, and it has opened the door to the development of innovative new strategies such as chimeric antigen receptor (CAR) T-cell therapy and oncolytic virus therapy. Due to the large variety of genes and mutations involved, CRISPR/Cas9's DNA repair tools can be used in a variety of situations, from base and primer editing to non-homologous end-joining and homology-directed repair. (Ugalde *et al.*, 2023).

A CRISPR/Cas12-based diagnostic technique for the quick and obvious detection of SARS-CoV-2 from extracted RNA from patient samples has been presented in recent papers (Broughton et al. 2020). These studies substantially increased the breadth of CRISPR/Cas9 applications by demonstrating the functional diversity obtained along multiple CRISPR/Cas evolution routes.

Methotrexate, a chemotherapy drug, is cytotoxic because it blocks the synthesis of nucleotides that the DHFR enzyme needs to create tetrahydrofolate (THF), which kills cells. Kanarek et al. created FTCD (formimidoyl transferase cyclo deaminase), an encoded enzyme needed for histidine catabolism, using a CRISPR/Cas9-based screening technique. They discovered that when numerous genes in the histidine catabolism pathway were missing, the susceptibility of cultured cancer cells to methotrexate was significantly reduced. Histidine may be introduced to the diet in order to increase the flow via the histidine breakdown pathway in vivo, which would make leukemic xenografts more susceptible to methotrexate (Kanarek *et al.*, 2018). Through the use of CRISPR/Cas technology in tumour metabolism, new knowledge has been discovered regarding the treatment of cancer (Zhang *et al.*, 2021).

3. In agriculture-

Crop breeding efforts could advance dramatically with the use of genome editing. Theoretically, this might encourage the creation of novel agricultural features that enhance productivity and pest resistance, promote climate change adaptation, and have applications in both the medical and industrial fields (Ricroch, A. 2019). Abiotic elements like salt, drought, and flooding, as well as climate change, pose severe hazards to agricultural output. As a result of climate change, abiotic stress is expected to worsen in agricultural systems. Gene editing can be a useful strategy for boosting crop resistance, according to recent research. Salinity and drought are two of the most important abiotic factors affecting rice, hence it is important to investigate the possibility of employing gene editing to create resistant varieties.CRISPR/Cas9 was used in one study to disable the salt-sensitive OsRR22 gene in rice (Karavolias *et.al.,* 2021, Zhang A. *et al.,* 2019).

4. In animals-

Genetic engineering has benefited a variety of animals, including cattle, pigs, carp, and sheep. This has sped up research and made previously impossible studies viable. Its use has improved animal welfare, disease resistance, cattle adaptability, and farming productivity. The nations working the hardest on animal genome editing include the United States, the United Kingdom, and China. To feed the world's expanding population, food output may need to rise by as much as 70%. Genome editing has the ability to decrease waste and increase the effectiveness of food distribution (Ricroch, A. 2019).

According to Bendixen *et al*., 2010, Chan, A. W. 2013, and Tsai *et a*l., 2007), large animals, particularly non-human primates (NHPs), pigs, and dogs, have numerous physiological, morphological, and metabolic characteristics in common with humans. They are perfect xenotransplant organ donors due to these similarities, and they also serve as models for human brain and cardiovascular diseases. After human disease-causing mutations were discovered, huge animals were utilised as disease models for pathology studies, medication development, and research into regenerative medicine.

There has been an increase in interest in generating and using genome-edited animals since the development of nuclease-mediated genome editing technology, which considerably broadens the range of genetic alterations that may be carried out in cattle. Thanks to improvements in cattle technology, advantageous genes that would have been lost through conventional breeding can now be maintained. This has also reduced the cost and production time for the necessary mutant animals. A unique breeding strategy for creating genetically modified organisms fit for human consumption has the potential to involve precise editing of the endogenous genome without the inclusion of foreign DNA (Zhao *et al.*, 2019).

The outcomes of Yang et al., who were successful in eradicating 62 copies of proviruses from the pig genome, were highly positive. The researchers created PERV-inactivated animals using CRISPR/Cas9 in a pig cell line and SCNT (Yang *et al.*, 2015, Niu *et al.*, 2017, Zhao *et al.*, 2019), which shows potential for the clinical application of PERV-free pig organs in xenotransplantation.

Challenges and possible concerns-

Clinical translation obstacles

Genome editing technologies face significant challenges that must be overcome, particularly those pertaining to the safety and efficacy of these treatments. Before these tools can be employed in clinical care, engineering progress in a number of areas will be required because these treatments' molecular makeup differs greatly from small-molecule and biologic treatments.

Raising gene correction's effectiveness

Although the amount of genomic change in a target cell population required to have a therapeutic effect varies from condition to condition, higher editing rates will increase the efficacy of most editing treatments. Editing is controlled, as indicated previously, by the rate at which DSBs are repaired. Given that NHEJ-mediated DSB repair is already present in most cell types and is rather effective, the main issue up to this point has been to increase HDR's effectiveness. Due to the fact that HDR's machinery is only selectively engaged during cell division and is downregulated in slowly cycling or post-mitotic cells, HDR has so far exclusively been used in dividing cells for genome editing.The ex vivo stimulation of mitosis using pharmacologic drugs has made it possible to largely avoidcell cycle regulation for slow-cycling cell types. The method has some promise, but it is unlikely to work with actual post-mitotic cells (Cox *et al.,* 2015).

Numerous cell line-based studies have revealed that during Cas9-mediated mutagenesis, the nuclease drastically changed a small number of off-target sites with a significant degree of sequence similarity to the target. Only off-target sites that could be predicted computationally are included in the research's conclusions. Recent whole genome sequencing of Cas9-edited cell lines revealed a low rate of off-target mutations, suggesting that the Cas9-mediated genome editing may be focused (Veres *et al.,* 2014). However additionally, the target selectivity can be improved by truncating the guide RNA or by using an RNA-guided FokI nuclease made by combining the catalytically inactive Cas9 domain with the FokI nuclease domain (Cox *et al.,* 2015).

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